

## THE PROBLEM OF THE ETIOLOGY OF HERPES ZOSTER.

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The nature and etiology of that group of infectious diseases of which one of the features is a vesicular eruption on the skin are at the present time much confused. Rivers (1) has constructed a table indicating a possible relationship between a series of these diseases beginning with sheep-pox and horse-pox and extending through cow-pox, smallpox, varioloid, alastrim, chicken-pox, and herpes zoster to symptomatic herpes and lethargic encephalitis. Certain of these conditions resemble each other in their clinical manifestations, others have little in common. Certainly, the symptoms of herpes simplex have little resemblance to those of smallpox. The only feature present in all of them, except lethargic encephalitis, is a vesicular eruption of the skin. In most of the conditions the skin lesions show similar histological characteristics.

At one time or another some relationship in etiology between various members of the group, or even an identical etiology in all of them has been suggested. In none of the conditions has the etiological agent been cultivated, but there is considerable evidence that the responsible agent in most of them is ultramicroscopic or filterable. It is evident that in the absence of cultivation, in order to establish the etiological relation of an ultramicroscopic virus with one of these diseases, it is necessary to reproduce, in animals or man, lesions resembling the natural infection. With certain of these diseases, notably smallpox and vaccinia, the experimental reproduction of the disease is comparatively easy. With others, such as varicella and herpes zoster, all attempts to transmit them to animals have led only to equivocal and uncertain results. In the case of herpes simplex, although a virus has been isolated which is highly infectious for rabbits, the clinical picture produced is not, as will be discussed below, identical with herpes simplex in man.

Recent investigations have suggested an especially close etiologic relationship between varicella, herpes zoster, and herpes simplex. Some of the workers, mainly on the basis of clinical observations, claim an identity of the etiological agent concerned in varicella and herpes zoster, others are of the opinion that herpes zoster is due to a modified herpes simplex virus. An etiologic relationship between these conditions is also suggested by the fact that in the epithelial cells of the skin lesions in all of them acidophilic intranuclear inclusion bodies are found. These bodies were first described by Tyzzer (2) in varicella and later by Lipschütz (3) in herpes simplex and herpes zoster.

Inclusion bodies, both intranuclear and extranuclear, have been studied in great detail by Lipschütz, and are considered by him to be characteristic of diseases of the filterable virus group. Goodpasture (4) has corroborated and extended Lipschütz's studies of these structures in connection with herpes simplex and agrees with Lipschütz as to the specific nature of intranuclear inclusion bodies. Rivers and Tillett (5) have demonstrated similar intranuclear inclusion bodies in the corium of skin lesions associated with a rabbit virus isolated by them. In the study of diseases of this group, the finding of these characteristic nuclear changes in experimental lesions in animals or man is therefore of importance in determining whether the reaction obtained is specific. Following the observation by Grüter (6) in 1920 and those of Löwenstein (7, 8) investigators in all parts of the world have demonstrated that the virus of herpes simplex, when inoculated into the scarified cornea of rabbits produces with great regularity a vesicular eruption followed by an intense keratoconjunctivitis. Inoculations into the skin less frequently give rise to lesions. As Doerr and Vöchting (9) first observed, corneal inoculations are frequently followed by marked nervous symptoms and death and similar symptoms can be produced by direct inoculation into the brain. The inoculations of the virus into rabbits, therefore, gives rise to lesions which *may* resemble those seen in man, but in most cases the lesions and symptoms differ both in character and severity from those present in the mild and common condition in human beings known as herpes simplex. It is of importance, however, that in all the lesions produced in animals, including those in the cornea, the skin, and the brain, the most characteristic feature of the lesion of herpes simplex in man is reproduced; namely, the occurrence of cells containing intranuclear inclusion bodies. The intracutaneous inoculation of the vesicular fluid of herpes simplex either into an individual already infected with herpes simplex or into a normal person has not given as constant results as has the inoculation of this fluid into the rabbit's cornea. Man's susceptibility to herpes simplex seems to depend on certain secondary factors which are, at the present time, unknown.

The successful inoculation of rabbits with herpes simplex material

was followed by attempts to transmit varicella to animals, but so far these attempts have not been successful. (For a review of the literature, see Rivers and Tillett (1).) Many attempts have been made to reproduce varicella in man by inoculating material from active cases into normal individuals. Kling (10) reported the successful vaccination of children against chicken-pox by inoculation with vesicular fluid. Certain later observers employing the method of Kling have noted the development of a local vesicle or papule at the site of inoculation, others have described the occurrence of a generalized eruption (true chicken-pox?), others have stated that no obvious lesions result from the inoculation. So far as we are aware no histological study has been made of any of the lesions described. The difficulty in successfully inoculating animals or man with varicella virus is of interest in view of the claims which have been made regarding the identity of the viruses of chicken-pox and herpes zoster.

*Inoculation of Virus of Herpes Zoster into Animals.*

Prior to 1921, attempts to inoculate animals with herpes zoster had proved negative. In this year Lipschütz (3) reported successful results.<sup>1</sup> Seven cases which were apparently typical as regards clinical manifestations were studied. The vesicular fluid was obtained early in the disease and was rubbed into the scarified cornea of rabbits. In certain instances the fluid was combined with the "roofs" of vesicles. Lipschütz considers that positive results were obtained with the material from four cases. In view of the importance of his conclusions a brief review of his cases will be given.

*Case I.*—It is stated that the inoculation of the material into the rabbit's eye was followed in 4 days by a slight opacity of the cornea along the lines of scarification. The eye was removed and sections were made through the cornea. Occasional giant epithelial cells were present along the lines of scarification. Under Bowman's membrane hypertrophied and swollen connective tissue cells

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<sup>1</sup> No attempt will here be made to review the entire literature concerning the inoculation of animals with material from cases of herpes zoster. Attention will be drawn chiefly to those authors whose results have been considered suggestive in contrast to those who have obtained definitely negative results. A complete review of the literature relating to herpes has recently been published by Doerr (34, 25).

were seen but no leucocytes. In the nuclei of the epithelial cells and also of the swollen connective tissue cells there were to be seen occasional, typical, round, sharply circumscribed and clearly demonstrable intranuclear inclusion bodies.

*Cases II and III.*—These were also early cases and material from them was similarly inoculated into the eyes of four rabbits. In one of the rabbits after 4 days the cornea showed an intense circumscribed keratitis, with the appearance of a slightly elevated “*eitrig getrüben*,” and “*daherweislich*” appearing vesicle. The microscopic examination of this eye showed marked infiltration with pus cells. No inclusion bodies were found. In another animal a keratitis developed, but the occurrence of vesicles or the presence of inclusion bodies is not noted. The results in the other two animals were negative. In these two cases, therefore, the evidence presented in the protocols which indicates positive results is very slight.

*Case IV.*—This was also a typical early case of herpes zoster. Vesicular material from this case was inoculated into the eyes of two rabbits and two guinea pigs. The results in the two guinea pigs and in one of the rabbits were negative. In the second rabbit the inoculated eye showed on the 2nd day conjunctivitis and circumscribed corneal infiltration. The eye was enucleated on the 4th day and in microscopical sections very numerous intranuclear inclusion bodies were found in the epithelial cells.

The remaining three cases were studied at later periods of the disease and the results were negative.

The experiments of Lipschütz with material from these seven cases can, therefore, as judged from his brief protocols, be considered to have yielded positive results in only two animals, and in these instances the results are of importance chiefly on account of the presence of intranuclear inclusion bodies in the epithelial cells.

Lipschütz (11) considers that his positive findings have been confirmed by Marinesco and Draganesco, Truffi, Mariani, and Blanc and Caminopetros, and that the successful transmission of herpes zoster to animals has thus been accomplished. It is therefore important to review in some detail the reports of these investigators.

Marinesco (12), and Marinesco and Draganesco (13), injected material from three cases of herpes zoster.

*Case I.*—Herpes zoster localized on the thigh. Vesicular fluid was inoculated into the scarified corneas of four small rabbits, and into the second cervical ganglion of two small cats. The rabbits all remained unaffected. The ganglia of the cats were examined after 7 days and in one of them there was evident lymphatic infiltration and atrophy of the neurones.

*Case II.*—Herpes zoster of the first and second branches of the trigeminus nerve. Since the vesicles contained but little fluid, spinal fluid obtained on the 6th day of the disease was used for inoculation. The injections were made into the anterior

chamber of one eye, and into the scarified cornea of the other eye in each of nine rabbits. Moreover, in five of these nine rabbits, in addition to the eye inoculations, 0.2 cc. of spinal fluid was inoculated intracerebrally. The eyes of the first three rabbits showed only injection due to injury. On the 4th day, in Rabbit 4, in the cornea of the eye in which the injection was made into the anterior chamber, there was noted a zone of infiltration. Rabbit 5 showed two points of infiltration on the scarified cornea. Rabbits 6, 8, and 9 were negative. Rabbit 7, besides a febrile reaction on the 4th day, developed an area of infiltration reaching the center of the pupil. The emulsified brain and cerebellum of this rabbit were inoculated into the cornea of four more rabbits, two of which showed on the 3rd and 4th days grayish infiltration along the lines of scarification. No statement is made concerning microscopic examination.

So far as can be judged from the protocols, therefore, inoculations made with the material from these two cases produced no characteristic lesions. A macroscopic infiltration of the cornea can hardly be regarded as specific.

*Case III.*—Herpes zoster lesions on the thigh. Vesicular fluid was inoculated into the scarified cornea of three rabbits, two of which on the 4th day showed a linear infiltration. The writer states (12) that the sections of the cornea of one of these rabbits showed swollen, edematous cells, and that here and there could be seen the "specific nuclear lesion, consisting in atrophy of the chromatin which is pushed toward the membrane, while the acidophilic mass, which has developed, offers a striking resemblance to the inclusions described by Lipschütz in animals injected with herpes." It is undoubtedly on this last statement that Lipschütz bases the view that his own observations have been confirmed by Marinesco and Draganesco. Certainly nothing else in the protocols indicates the occurrence of a specific lesion.

Truffi (14) studied three cases of herpes zoster. The results in the first two were negative. Vesicular fluid from the lesion of Case III, cervicobrachial in distribution, was obtained on the 3rd day of the disease and inoculated into the scarified cornea of one rabbit. After 48 hours a slight opacity along some of the lines of scarification, and an intense conjunctivitis were noted. The corneal opacity disappeared rapidly and the eye regained its normal appearance by the 7th day. 22 days after inoculation the rabbit showed symptoms of encephalitis and was killed 10 days later. The microscopical examination of the brain was negative. The presence of intranuclear inclusion bodies in the brain cells is not noted. The inoculation of the brain emulsion into the scarified corneas of two rabbits and two guinea pigs failed to produce lesions.

Most of the attempts made by Mariani (15) to inoculate the cornea of rabbits with herpes zoster resulted negatively. In one instance he obtained a very acute keratoconjunctivitis with hypopyon and purulent ophthalmia. In only one case did there result a keratitis which he was able to transmit in series. The lesion produced was clinically and symptomatically very similar to the keratitis produced by herpes simplex virus. No description of the case of herpes zoster from which

the material for inoculation was obtained, is given. No statement concerning microscopic examination of the corneas is made. Mariani himself considers this single experiment inconclusive.

Material from nine cases of herpes zoster was inoculated by Blanc and Caminopetros (16) into the eyes, cornea, conjunctiva, skin, brain, and spinal cord of a series of animals, including rabbits, mice, sheep, pigeons, monkeys, and a dog. Three monkeys (*Macacus rhesus*) were inoculated as follows: one into the eye, one into the spinal canal, and the third into the skin of the thoracic region which had previously been shaved and excoriated. The inoculations in the first two monkeys resulted negatively. The third monkey showed a slight inflammatory reaction at the site of inoculation but recovered without the appearance of vesicles. All the other animal experiments gave negative results with the following exceptions; two rabbits developed a late paralysis which, however, the authors considered was probably not specific, and one rabbit and one sheep, both inoculated with material from the same case, developed a definite keratitis, which spread from the point of inoculation. The writers think that this lesion might easily be interpreted as a reaction resulting from the injection. They conclude that the problem of the transmission of herpes zoster to animals remains open and they apparently consider their own experiments negative or inconclusive.

Meineri (17) claims to have produced encephalitis in a guinea pig by the intracerebral inoculation of vesicle fluid from a case of herpes zoster. A careful analysis of his experiments, however, in our opinion, indicates that his findings can best be interpreted as the result of trauma. The writer also injected vesicle fluid obtained on the 3rd day of the disease from one of his cases of herpes zoster into the skin of the arm of the patient and into the skin of a normal man. These injections in both instances were without visible result.

The review of the publications of those writers whom Lipschütz quotes as having confirmed his work shows that two of the writers regard their own results as inconclusive. Only Marinesco and Draganesco found microscopical lesions which might be interpreted as specific.

On the other hand, many other authors report entirely negative results following the inoculation of herpes zoster material into the sacrificed corneas of rabbits: Kraupa (18); Baum (19); Löwenstein (8); Teissier, Gastinel, and Reilly (20); Kooy (21); Netter and Urbain (22); Bloch and Terris (23); Simon and Scott (24); and Doerr (25).

It is evident, therefore, that the results of attempts to inoculate animals with material from cases of herpes zoster must be considered at present to be inconclusive.

*Herpes Simplex and Herpes Zoster.*

Although it has not been possible to demonstrate conclusively any specific virus associated with herpes zoster, certain writers have presented evidence which suggests that, in certain cases at least, the symptoms and lesions of herpes zoster may result from the presence of the virus of herpes simplex.

Luger and Lauda (26) have published several papers on the problem of the etiology of herpes zoster. In their first paper they give the results obtained by inoculation with material from seven cases of typical herpes zoster, employing the technique used by Lipschütz. In none of the eyes inoculated did any macroscopic reaction occur. On microscopical examination there was found fairly regularly edematous swelling of the epithelial cells, giant cell formation, and "ballonierende" degeneration, but in no instances were cell inclusions or characteristic changes of the nuclei seen. They themselves considered the results in this series of experiments negative. This paper was followed by two others (26) in which two more cases, considered by the authors to be typical cases of herpes zoster, were studied. From both of these latter cases these investigators were able to isolate a virus, infectious for rabbits, which they showed, by immunity experiments, to be identical with the virus of herpes simplex. The first case occurred in a patient suffering from acute epidemic encephalitis, and the eruption was localized in the gluteal region. The involved area was not large and the clinical diagnosis of herpes zoster in this case must be considered doubtful. A typical herpes simplex virus was isolated without any difficulty. In the second case the eruption was thoracic in distribution and the case was clinically typical. On the 8th day of the disease, some vesicle fluid was inoculated into the eye of one rabbit. No local reaction occurred. The 10th day after inoculation the animal developed encephalitis and died on the 11th day. For reasons not stated, the glycerolated brain of this animal was stored on ice for 11 days before further inoculations were made. Inoculations then made with this material into the brains and eyes of rabbits produced the characteristic reactions of a herpes simplex virus. The authors conclude from these results that herpes simplex virus may be isolated from herpes zoster vesicles. They offer no explanation of the fact that although the virus failed to produce any reaction on the cornea, it nevertheless possessed the ability to invade the brain.

Grüter (27) inoculated material from three cases of herpes zoster into the scarified corneas of rabbits. A mild keratitis resulted. No detailed description of the lesion or results of microscopic examination are given. Grüter, however, believes the lesion obtained was specific and attributes it to herpes simplex virus of a low grade of virulence. He states that there is no evidence for assuming a specific virus for herpes zoster. The data presented, however, are not sufficient to establish the isolation of a true herpes simplex virus from these cases.

Bastai and Busacca (28), in a general article on herpes, state that they inoculated material from three cases of herpes zoster into the cornea of rabbits and into the cornea of one monkey (*Macacus*). Rabbits were also inoculated intracerebrally. None of the animals showed any reaction, with the exception of one rabbit which developed a slight keratitis. No attempts were made to transmit this lesion, and no microscopical examinations are reported. These authors also are of the opinion that herpes zoster is probably a manifestation of infection with herpes simplex virus. The experimental data presented, however, are hardly sufficient to justify this point of view.

Cipolla (29) is of the opinion that the cases of herpes zoster should be divided into two groups, zoster symptomaticus and zoster idiopathicus, although he states that there is great difficulty in drawing any sharp line of division. He has studied four cases which he considered zoster idiopathicus and three cases which he called zoster symptomaticus.

The results of inoculation of material from the so called idiopathic cases were as follows: In one rabbit and two guinea pigs there occurred "a keratitis definitely evident along the lines of scarification but without vesicle formation and a hyperemic conjunctivitis, with redness and slight seromucous exudation." The results in all the other animals inoculated were negative. No note is made of any microscopical examinations.

Of the cases which he considered symptomatic, in one the eruption followed an injury, in the second it was associated with a tuberculous pleurisy, and in the third the patient was taking bromides and the herpetic eruption was considered to be a manifestation of bromide intoxication. In all these cases the eruption was on the chest. In two cases the appearance of vesicles was preceded by acute neuralgic pains, in the third case no note is made concerning pain, but the eruption was accompanied by a febrile reaction. The inoculations with material from all these cases gave rise to severe keratitis and conjunctivitis resembling that caused by the virus of herpes simplex. In one rabbit an encephalitis developed. No statement concerning microscopical examinations is made.

In our opinion the three so called symptomatic cases must be considered from the clinical features as probably cases of herpes zoster. The comparatively trivial associated injuries (trauma or drug) might easily have been overlooked and then the diagnosis would not have been questioned. It is of great interest that the animal experiments indicate that in these cases a virus indistinguishable from that of herpes simplex was present in the lesions.

Teague and Goodpasture (30) were able to produce zoster-like lesions in the skin of rabbits and guinea pigs by the inoculation of herpes simplex virus into areas of the skin previously treated with coal tar. The study of the corresponding posterior root ganglia showed lesions comparable to those found in man in the ganglia innervating the area of zonal eruption.

The writers do not maintain that they have reproduced the human disease herpes zoster in animals, but they believe there is a close analogy between the experimental condition produced by them and true herpes zoster. In their

opinion the herpes simplex virus first multiplies at the site of inoculation in the skin and passes up the corresponding spinal nerve to its spinal ganglion; the virus then seems to pass centrifugally along the nerve and its branches to the skin, where it multiplies rapidly and gives rise to characteristic herpetic vesicles. They draw attention to the difficulty of sharply separating cases of herpes simplex and herpes zoster and discuss the occurrence of intermediate cases. From a case of the intermediate type inoculations were made into the tarred skin of a rabbit. A zonal eruption, as described above, resulted. They feel that the evidence presented strongly suggests that the virus of herpes zoster is closely allied to the virus which causes herpes simplex, probably differing only in virulence.

The interesting hypothesis presented by Teague and Goodpasture (30) and by Luger and Lauda (26) concerning the relation of herpes zoster to herpes simplex does not find acceptance, however, by Lipschütz (31). He emphasizes the point of view that in the production of an experimental herpes zoster it is of prime importance that the starting point be a typical clinical case and not a border line case.

At the present time the evidence that herpes zoster may result from infection with herpes simplex virus rests upon the isolation of a virus apparently identical with that of herpes simplex from a small number of cases. No description of the type of case from which the material employed for inoculation was obtained is given by Grüter or by Bastai and Busacca. The case described by Teague and Goodpasture and the first case described by Luger and Lauda belong to the intermediate type of cases. The second case of Luger and Lauda, and the so called symptomatic cases of Cipolla, seem to have been clinically typical cases of herpes zoster. It is possible, therefore, that in certain instances the virus of herpes simplex may be isolated from cases clinically characteristic of herpes zoster, but the evidence for this is not complete and the conclusion that herpes zoster may be the result of infection with herpes simplex virus needs further verification.

#### *Varicella and Herpes Zoster.*

Several observers (Lipschütz, Meineri, and others) have made isolated attempts to inoculate human volunteers with herpes zoster, but always with negative results. Recent studies of Kundratitz (32) seem to show that herpes zoster can be successfully transmitted to very young children. This author wished to test out von Bókay's (33) hypothesis, based on clinical observation, that the virus of varicella, under certain unknown conditions, may produce a typical picture of herpes zoster and that the virus from this lesion may in turn cause varicella. He therefore

attempted to immunize children against varicella by the inoculation of material from herpes zoster cases. His first results were negative, but his later attempts proved successful. He now reports that he has inoculated material from ten typical cases of thoracic herpes zoster and has had positive results with the material from five of these cases. Positive reactions were obtained only in children under 5 years of age. Children who reacted positively were subsequently shown to be immune to varicella.

Kundratitz's work seems to indicate that the virus of varicella and that of herpes zoster are identical or, at least, closely related. It is unfortunate that Kundratitz does not give a description of the cases of herpes zoster used by him for inoculation. It would be interesting to know whether there were any clinical differences between the five cases of herpes zoster with which he was able to make successful transfers and the five cases in which transfers resulted negatively for, as von Bókay and others have shown, the vesicles of varicella may be quite localized, resulting in lesions resembling herpes zoster. The relation between herpes zoster and varicella will, in all probability, not be entirely cleared up until we are able to transmit either one or both of these diseases to animals.

#### EXPERIMENTAL.

Nine cases of herpes zoster have been studied by the writers and inoculations have been made into a series of animals. The following are abstracts of the histories and protocols of the experimental studies.

*Case I.*—A. M. Age 13. Patient admitted to the hospital Oct. 4, 1924, suffering from subacute rheumatic fever and chronic cardiac disease. She gave no history of a previous attack of herpes zoster or chicken-pox. The arthritis had almost entirely disappeared and the cardiac lesion was well compensated, when on Dec. 6, 1924, the patient complained of pain and itching over the upper scapular area, in the axilla, and posterior part of the upper arm. On examination of this area there was discovered a rash consisting of small, discrete papules and vesicles distributed in patches over a zone on the upper chest from the midline behind to the midsternal line in front, and over the inner and posterior surface of the arm. The area of distribution corresponded to Head's second and third dorsal areas. During the following days the vesicles became larger. The temperature was not higher than 99.8° until Dec. 11, when some of the vesicles had become pustular, and now the temperature rose to 101.4°. The pain was severe and characteristic of herpes zoster, and the appearance and distribution of the lesions were typical. A small piece of skin was removed and microscopical sections showed characteristic vesicles with numerous intranuclear inclusion bodies in the epithelial cells.

On Dec. 9, the 3rd day of the disease, fluid was pipetted from a number of vesicles and a small piece of the involved skin was obtained. The skin was ground between two glass slides, the ground material was washed off in a small amount of normal saline solution and was added to the vesicular fluid. Small amounts of this emulsion were rubbed into the scarified<sup>2</sup> corneas of two rabbits, Nos. 1 and 2, and also injected intracutaneously into the shaved skin of Rabbit 2 and into the skin of Guinea Pig 1. The area of skin in the guinea pig where the injection was made had been painted several days previously with coal tar solution. The emulsion was also rubbed into the scarified skin of Rabbit 3, which had received one painting of tar 5 days before, and into the scarified skin of a similarly tarred guinea pig, No. 2. (The rabbit and guinea pigs were painted with a refined coal tar solution obtained through the courtesy of Dr. Jas. B. Murphy. This refined coal tar was much less toxic than ordinary tar and could be applied in a fairly thick coat, so that one painting resulted in a marked reaction.) On the following day, Dec. 10, vesicular fluid was again obtained from fresh vesicles and also another piece of skin. This material was treated in the same way as that obtained on the preceding day, and inoculated in the following ways. The cornea of Rabbit 1 and the scarified tarred skin of Rabbit 3 and Guinea Pig 2 were reinoculated. Some of the material was also inoculated intracerebrally into Rabbit 4 and into the scarified cornea of Rabbit 5. Thus, with fresh material obtained on the 3rd and 4th days of the disease, five rabbits and two guinea pigs were inoculated in various ways.

The animals were carefully observed each day following the inoculations, but in none of the animals were any macroscopic changes seen which could be ascribed to the inoculations. One of the eyes of Rabbit 2 was removed on the 3rd day and the other on the 7th day following the inoculations and sections were made through the corneas. The sections show in places what are apparently the results of mechanical injuries and in the section of the eye removed on the 7th day, foci of slight infiltration of the substantia propria with small round cells. Some swelling of certain of the epithelial cells is also seen. But nowhere are there any signs of vesicle formation or marked inflammatory reaction and no inclusion bodies were found.

Although no definite reactions were obtained in this first series of animals it was thought that by inoculating from one cornea to another and from one brain to another, the virus might possibly become adapted to the rabbit and produce definite lesions in subsequent transfers. Therefore, starting with Rabbit 1 inoculations were made from one rabbit to another by scraping the cornea and washing out the conjunctival sac with a small amount of saline and inoculating the material thus obtained into the scarified cornea of another rabbit. Fourteen corneal passages were thus made, at 2 and 3 day intervals. In many of the rabbits, the scarified eye on the day following the inoculation showed a slight degree of opacity along the lines of scarification and a slight exudation. However, it was found

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<sup>2</sup> The eyes of the rabbits were anesthetized locally with cocaine before inoculation. For other operations the animals were given ether.

during the course of the study that slight changes of this character frequently occur following the inoculation of an emulsion of normal rabbit cornea, and even after scarification alone without the injection of any foreign matter whatever. Except for these slight non-specific reactions no changes were observed in any of the eyes of the series. In certain instances, although no gross changes were present, the cornea was sectioned but no lesions which could be considered specific and no intranuclear inclusion bodies were found.

Starting with Rabbit 4 inoculated intracerebrally with the material from this case, ten brain to brain transfers were made at 5 day intervals. Each animal was killed with ether, and the brain removed with sterile precautions. An emulsion of the brain was made with Locke's solution in a sterile mortar, the suspension centrifuged at low speed, and 0.2 cc. of the supernatant fluid injected intracerebrally into a normal animal. At the same time, some of the brain emulsion was inoculated intracorneally and intradermally into each of two other rabbits. It was found that the inoculation of brain emulsion into the scarified cornea usually was followed by conjunctivitis of considerable severity which, however, proved to be wholly non-specific. The temperature of the intracerebrally inoculated rabbits was taken daily, and sections of the brain of each of the inoculated animals made. None of the rabbits showed a significant rise in temperature and careful study of the brain failed to reveal any characteristic lesions. No intranuclear inclusion bodies were found.

*Case II.*—F. T. Age 13. Patient admitted to the hospital Nov. 13, 1924, suffering from rheumatic fever and cardiac disease. History unimportant in the present connection except that the patient stated that he had had chicken-pox. On the afternoon of Feb. 1, 1925, the patient showed a definite rise in his pulse rate (120). This was attributed to the excitement due to visitors and playing a game. There was no rise in temperature. On Feb. 2 there was discovered a vesicular (herpetiform) eruption over a small area on the lower right chest midway between the nipple and the costal margin. He did not complain of pain or itching and there was no constitutional disturbance. On Feb. 4 when the vesicles were already beginning to become dry, the patient complained of slight itching. A small piece of skin where the rash was present was removed at this time. A portion of this was cut into sections and showed characteristic multilocular vesicles, with swollen epithelial cells containing intranuclear inclusion bodies.

This patient was in the hospital at the same time as Patient I. The two patients were at opposite ends of the same floor, but in different wards. Case II developed nearly 2 months after Case I, and it is not likely that there was any direct connection between them.

The lesion in Case II was so slight that it was thought the condition might be that described in the literature as zosteriform herpes rather than true herpes zoster. The distribution of the vesicles was suggestive of herpes zoster, but the vesicles remained small, the patient never complained of pain in the affected area, and there was no fever.

In view of the clinical course in this case and the somewhat uncertain diagnosis

it was decided to inoculate all the material obtained into the corneas of rabbits. If the lesion were due to herpes simplex virus which had accidentally become implanted on the thorax, the inoculation of the material from this case into the scarified corneas of rabbits should produce typical keratitis. If, on the other hand, the condition were true herpes zoster, it would be advisable to employ as nearly as possible the technique used by Lipschütz. Consequently the piece of skin removed from the affected area was ground up in a mortar without sand in a small quantity of normal saline solution. Portions of the suspension were then rubbed into the scarified right corneas of six young rabbits. Cocaine was used instead of ether to avoid lacrymation. After inoculation the eye was held in position for a short time so that the inoculum might dry on the cornea. The left cornea of each animal was scarified in the same way as the right and left uninoculated to serve as control.

Daily comparisons were made between the inoculated corneas and the corneas which had merely been scarified. In some of the animals a slight conjunctivitis was observed in one or the other eye. But whereas in one instance the reaction seemed more intense in the inoculated eye, in another the control eye appeared worse. In none of the animals did a severe keratitis develop. The possibility that the infective agent in this case might be herpes simplex virus was, therefore, in all probability excluded.

On the 4th day after inoculation although no definite macroscopic lesion developed, the right eyeball of each of the six rabbits was removed, fixed in Zenker's solution, and sectioned. Careful examination of the sections of the six corneas of the inoculated rabbits failed to show any definite lesions or the presence of intranuclear inclusion bodies.

*Case III.*—J. L. Age 16. The patient was admitted to the hospital Oct. 5, 1924, suffering from diffuse glomerular nephritis. The patient stated that neither he nor any other member of his family had had chicken-pox. On Feb. 11, a vesiculopapular eruption appeared on the lateral and anterior aspects of the left side of the lower chest and upper abdomen, extending over the lumbar region to the midline behind. The eruption occupied the region corresponding to Head's eighth dorsal area and was typically that of herpes zoster. It is of interest that in this patient there appeared on Feb. 17, on the right or opposite side, over the abdomen and flank, a vesicular eruption. The eruption here was exactly like that on the opposite side but much less extensive, and also had a zonal distribution, but slightly lower than on the left, corresponding to Head's tenth dorsal area. Bilateral distribution of the rash in herpes zoster is rare though it does occur, as in this case. This patient occupied a bed directly opposite that of F. T., Case II, and the rash first appeared just 10 days after that of F. T. As herpes zoster is extremely uncommon in this hospital it seems reasonable to suspect some connection between these two cases and also some relation between these cases and Case IV. On the day after the rash appeared a small piece of skin where the rash was present was removed and a little vesicle fluid was obtained. At this time most of the rash was still in the papular stage. A portion of the skin was sectioned and the

examination showed the typical lesions of herpes zoster with many cells containing intranuclear inclusion bodies.

The skin removed on Feb. 12 was ground up in a mortar with Locke's solution and combined with the vesicle fluid. The material was centrifuged at low speed for a few seconds to throw down the larger particles, and about 1.5 cc. fluid was obtained. Monkey 1 (*Macacus rhesus*) was inoculated with this fluid in the following ways: intracerebrally, intracutaneously, into the scarified cornea, and directly into the anterior crural nerve. No reactions occurred at the sites of the corneal and intracutaneous inoculations. No nervous or other symptoms developed. The monkey later developed tuberculosis and was killed on Mar. 9.

On Feb. 14, 1925, the 3rd day of the disease, the "roofs" of four more vesicles were removed, ground up without sand, and inoculated into the scarified corneas of six rabbits. Some of the same material was also inoculated into the scarified skin on the arm of a human volunteer whose history was negative for chicken-pox and herpes zoster. The result of the latter experiment was negative. Three of the inoculated rabbits were bled to death after 24 hours, the eyeballs removed and fixed in Schaudinn's solution and stained with Giemsa. The other three rabbits were observed for 72 hours, the eyeballs removed and treated in the same way. The sections of the corneas of these six rabbits showed no lesions other than those resulting from the scarification. No intranuclear inclusion bodies were found.

*Case IV.*—R. N. Age 37. This patient developed the lesions of herpes zoster on Feb. 22, 1925. He occupied a bed in the same ward as Patients II and III, and the rash appeared 11 days after that of Case III. The patient was an adult man, 37 years of age. He was admitted to the hospital on Dec. 12, 1924, suffering from diffuse glomerular nephritis. He stated that as far as he knew he had never had chicken-pox. On Feb. 22, 1925, a vesicular eruption appeared over a zone extending over the right lumbar region posteriorly and just below the umbilicus anteriorly. The area of distribution corresponds roughly to Head's tenth dorsal area. The rash was not preceded by any subjective symptoms and was not noticed by the patient until pointed out to him by another patient. The patient seemed quite comfortable on this date. On Feb. 23 the eruption became more marked, and groups of large, tense vesicles were present. Considerable hyperesthesia developed in the involved area. The patient's temperature rose to 101.6° and he complained of general malaise. On the following days he complained of pain, both superficial and deep, along the course of the involved nerve root. The deep pain was of a sharp, cutting character, and paroxysmal in type, and was referred to the right iliac region. Some of the larger vesicles later became hemorrhagic and an enlarged, very tender axillary lymph node appeared on the affected side.

On Feb. 24, the 3rd day of the disease, some vesicle fluid was collected in a sterile syringe containing a trace of sodium oxalate solution. The fluid was injected into the spinal cord of Monkey 2 (*Macacus rhesus*). The monkey was shaved over the dorsal region on both sides so that in case a rash developed it would be

visible. Some of the vesicle fluid clotted before all the injections could be made and the clot was placed in a mortar and ground up with a small amount of salt solution. This material was injected intracerebrally and intracutaneously into Monkey 2, and a small amount was also rubbed into the scarified cornea. The results of the skin and eye inoculations were completely negative. No cutaneous reactions appeared at the level of the spinal cord inoculation. The monkey never showed any nervous or other symptoms. No rise in temperature was observed.

On Mar. 28, a month after the inoculation with herpes zoster material, the monkey's susceptibility to herpes simplex virus was tested by intracerebral inoculation of a virulent herpes simplex virus. The monkey never showed a febrile reaction or any other symptoms.

On Feb. 24 vesicle fluid from the patient was obtained and immediately rubbed into the scarified cornea of a rabbit. The rabbit showed a slight amount of conjunctivitis after 24 and 48 hours, but the eye appeared normal after 72 hours.

On Feb. 28 the "roofs" of four vesicles were removed. This tissue was ground without sand, mixed with a small amount of saline, and the suspension was injected intracranially into two rabbits and intraspinally into two others. The rabbits which received the injections into the cord were shaved at the level of inoculation so that any skin reaction would be visible. All four of the rabbits remained well, and never showed any febrile or other reaction.

*Case V.*—E. K. Age 19. Student. The patient was admitted to the hospital May 23, 1925, with the diagnosis of herpes zoster. The past history is unimportant except that he is sure that he had chicken-pox at the age of 5. On May 18, 5 days before admission, he first noticed a slight dull pain in his chest on the left side, and this pain persisted. On May 20 the pain became much worse, and an eruption of "small blisters" over the left breast, anterior to the axilla, was noted. He had slight fever and slight general malaise. He described the pain as being a "stinging itch;" sometimes it became a dull, constant pain. The pain was present not only over the area involved in the rash, but radiated to the lower left back. On admission there was present on the left side of the thorax an irregularly arranged, rather sharply delimited, papulovesicular eruption. The eruption occupied, roughly, a horizontal zone 6 to 8 cm. broad, just below the level of the nipple, occupying Head's fifth dorsal area. The vesicles varied in size, and were mostly discrete. There was slight tenderness over the affected area and there was some hyperesthesia of the uninvolved skin in this area. A small piece of the affected skin was removed on May 23. Microscopical sections of this showed typical vesicles of herpes zoster with numerous intranuclear inclusion bodies. At the same time three other small pieces of skin were obtained and ground without sand in a sterile mortar in a small amount of Locke's solution. 0.2 cc. of the supernatant fluid of this emulsion was injected into each testicle of a young vervet monkey (*Cercopithecus lalandii*), commonly called the green monkey. The monkey's temperature remained normal on the succeeding days. On the 4th day after inoculation the left testicle was removed. No gross changes were evident. The whole testicle was placed in Zenker's solution and the following day was divided

into five blocks and sections were cut from each block. On the 5th day after inoculation the right testicle was removed and treated like the left. Numerous sections from both testicles were studied and, although the left testicle seemed to show a definite lesion with hemorrhage and necrosis, no typical intranuclear inclusion bodies were found in sections from either testicle.

The attempt to inoculate a vervet monkey with material from this case was, therefore, unsuccessful. There is still disagreement among the various investigators as to whether monkeys are susceptible to herpes simplex virus. An attempt was made to infect this monkey later with fresh virulent herpes simplex virus but no reactions occurred. This species of monkey is therefore probably insusceptible or at least not highly susceptible to herpes simplex virus.

*Case VI.*—P. C. Age 41. The patient was admitted June 30, 1925. He was uncertain whether or not he had had chicken-pox. On the evening of June 25, 1925, when the patient was walking home from work he felt an intense itching sensation around the right scapular region, extending anteriorly around into the right axillary region. Later in the evening small vesicles began to appear in this area. The rash appeared over the right scapula and anteriorly below the right mammary region, the distribution corresponding to Head's third and fourth dorsal areas. There was considerable pain in the involved regions.

On June 30, on the 6th day of the disease, four small pieces of skin were removed and ground in a sterile mortar without sand in a small amount of Locke's solution. 0.1 cc. of this emulsion was injected into each testicle of a young vervet monkey. On the following day, July 1, 1925, the monkey's temperature showed no striking rise. Both testicles were enlarged. 24 hours after inoculation the left testicle was removed. The whole testicle was placed in Zenker's solution, and the next day was divided into three blocks. July 2, the right testicle was removed and treated in the same way. Numerous sections from both testicles were studied, but no specific lesions with intranuclear inclusion bodies were found.

*Case VII.*—J. S. Age 38. The patient was admitted July 2, 1925. The patient has syphilis and had received antisyphilitic treatment at the Presbyterian Hospital since March. She did not know whether or not she had had chicken-pox. For 2 or 3 days before admission she had pain over her left breast and over the left side of her back in the scapular region and in her left arm. On July 1, small vesicles appeared on her back and on the left breast, and a fairly large crop of vesicles appeared in the axillary region. The distribution corresponded to Head's fourth dorsal area.

On July 2, 1925, the 2nd day of the disease, some vesicle fluid was collected from the larger vesicles, and seven small pieces of skin were removed and ground up without sand in a small amount of Locke's solution. The vesicle fluid was added to the skin emulsion, and 0.2 cc. of this material was injected into each testicle of a young vervet monkey. An intracutaneous injection was also made. The following day the testicles appeared enlarged. 40 hours after inoculation the right testicle was removed and sections made. 72 hours after inoculation the left testicle was removed and sections made. Numerous sections of both

testicles were studied, but no specific lesions with intranuclear inclusion bodies were found. The result of the intracutaneous inoculation was completely negative.

Inasmuch as this patient was receiving injections of salvarsan and mercury, it was thought that this case, in spite of the typical clinical course and characteristic distribution of the vesicles, might represent an example of so called symptomatic herpes zoster described particularly by Cipolla and in the cases of which he found the virus of herpes simplex. Tests were therefore made for the presence of herpes simplex virus by inoculating some of the material from this case into the eyes of four rabbits, but the results were negative.

*Case VIII.*—S. B. Age 14. The patient was admitted July 13, 1925. The patient had been in the hospital since May 22 suffering from pleurisy. She stated definitely that she remembered having had chicken-pox at the age of 6 or 7 years. On July 12 she complained of pain in her right shoulder and on examination a vesicular rash covering the right scapular region was found. The eruption extended from the lower half of the wing of the scapula to the vertebral column. A few isolated vesicles were present anteriorly to the right of the nipple. The area involved corresponded to Head's fourth dorsal segment. A slight hyperesthesia of the skin was noted.

On July 14, the 3rd day of the disease, three fairly large pieces of the involved skin were obtained and ground up without sand in a small amount of Locke's solution. Injections of this material were made in the following ways. It was injected into the testicles of a young vervet monkey, intracutaneously into the scarified skin of one tarred guinea pig and one tarred rabbit, and into the scarified corneas of two rabbits. The inoculated eyes and skin remained completely negative. On July 15 the monkey's temperature was 103°. On July 16 the temperature was 100.6° and the right testicle was removed for section. On July 17 the left testicle was removed. Numerous sections of both testicles were studied but no specific lesions with intracellular inclusion bodies were found.

*Case IX.*—H. S. Adult male. The patient had had chicken-pox in childhood but had never had herpes zoster and had not recently been in contact with any case of herpes zoster. His wife had had a herpes simplex eruption a short time previously. The papular rash over the right shoulder appeared after 2 weeks of slight indisposition and after several days of severe headache and pain in the back.

On the 1st day of the eruption the tops of three papules were removed, immediately ground without sand, and injected into both testicles of a young vervet monkey. There occurred no febrile reaction and on the 3rd day the left testicle was removed and on the 4th day the right testicle was removed. Evidences of injury with hemorrhage were seen but on section no marked cellular reactions were present and no inclusion bodies were found. Some of the material from this case was also inoculated into the scarified corneas of two rabbits with negative results.

## SUMMARY AND DISCUSSION.

Attempts were made to produce lesions in animals by the injection of material obtained from the vesicles and involved skin of nine cases of herpes zoster. All the cases, with the exception of one (Case II), were characteristic cases of idiopathic herpes zoster and the question of their being cases of so called zosteriform herpes or symptomatic herpes zoster can hardly be raised. As regards Case II, if this case occurred alone, there might be some doubt as to its nature on account of the mildness of the symptoms and the small area of skin involvement. Taken in connection with Cases III and IV, however, which occurred in the same ward and in patients who were quite closely in contact with Patient II, it seems fairly reasonable to assume that they were all of the same character. Cases of herpes zoster have been extremely rare in this hospital and the occurrence of three cases in the same ward within a very short period of time suggests very strongly a transference of infection from one case to the other. That Case II was not one of herpes simplex also seems fairly certain from the negative results obtained by inoculation of rabbits' eyes with vesicle material.

In making the animal experiments we employed various methods which were suggested largely by the technique used by previous observers, especially by those who have reported results which were considered positive. In making inoculations into the corneas the technique recommended by Lipschütz was employed as far as possible. Young rabbits were used and the material was obtained from fresh vesicles early in the disease and inoculated with as little delay as possible. The material injected into rabbits' eyes was obtained from seven cases and twenty-four rabbits were used. In judging of the results obtained in this kind of experimentation great caution must be observed. Our experience convinces us that slight opacities occurring along the lines of scarification and mild conjunctivitis cannot be held to indicate the effect of a specific virus. As regards the interpretation of the microscopic changes found, we were quite familiar with the appearance of intranuclear inclusion bodies as seen in the lesions of experimental herpes simplex and the filterable virus (Virus III) indigenous to rabbits described by Rivers and Tillett (5). We

also had no difficulty in finding intranuclear inclusions in the sections of skin removed from patients. It is not likely, therefore, that these structures were overlooked in our study of the sections. Briefly stated, although the material studied was satisfactory and in spite of the fact that a considerable number of animals were used for each case, we have been unable to confirm the observations of Lipschütz regarding the experimental production of specific lesions in the corneas of rabbits. We realize that this is only negative evidence and therefore not of conclusive importance in view of Lipschütz's observations. It indicates, however, that the production of specific lesions in rabbits' eyes with material from herpes zoster vesicles is extremely difficult and that successful results may be a matter of chance, depending, possibly, on peculiar susceptibility on the part of the rabbits. In view of the fact, however, that a careful analysis of the positive results reported by other observers shows that the conclusions were based on insufficient evidence, we believe that further work is necessary before the successful inoculation of the rabbits' corneas with herpes zoster virus can be accepted as fully demonstrated. To make the evidence convincing specific lesions should be obtained with a fair degree of regularity and the virus should be successfully transmitted through at least two generations. Apparently the latter was not attempted by Lipschütz.

Intracerebral inoculations into three rabbits with material from two cases (Nos. I and IV) were made. Two rabbits were also inoculated intraspinally with material from one case (No. IV). None of these animals showed any reaction. In the case of one of the animals inoculated into the brain (Case I) although this rabbit showed no symptoms, we thought it conceivable that the susceptibility of the species for the virus might be so slight that no obvious lesion had been produced. Nevertheless it was thought that the virus might possibly remain alive at the seat of inoculation and by repeated transfers become adapted to the rabbit. This phenomenon has been observed by Noguchi with vaccine virus, and by Rivers and Tillett with the rabbit virus isolated by these workers. This possibility was tested by us by making serial corneal and brain inoculations. Corneal transfers were carried through fourteen animals in series, and brain transfers through ten. No specific lesions developed in any of the animals.

The work of Teague and Goodpasture suggested that the skin might be rendered more susceptible to infection by previous treatment with tar. Material from two cases (Nos. I and VIII) was inoculated into the tarred skin of guinea pigs and rabbits. The material was injected intracutaneously and also rubbed into the scarified skin. No reaction was obtained in any of the animals.

Finally, the transmission of herpes zoster to monkeys was attempted. Blanc and Caminopetros, and Bastai and Busacca, as discussed in the review of the literature, inoculated monkeys (*Macacus*) in various ways, without success.

It was thought possible that although monkeys of the genus *Macacus* might be refractory, monkeys of another genus might prove susceptible. Consequently, besides the inoculation of two *Macacus* monkeys, attempts were made to infect five vervets. Moreover, in view of the fact that the virus of vaccinia and the rabbit virus of Rivers and Tillett could be successfully cultivated in the testicle, intratesticular inoculations were employed. The testicles were removed at varying periods following inoculation. Numerous sections of these testicles were made and examined, but in no instance were any lesions found which could be interpreted as specific. No cells containing intranuclear inclusion bodies were found. These experiments, therefore, have also led to purely negative results.

This report of our work is made at the present time because a considerable amount of literature has been published which gives the impression that herpes zoster has been successfully transmitted to animals. Although the observations of Lipschütz are suggestive, it is important that they be confirmed by further investigations.

Until herpes zoster can be regularly transmitted to animals and cross-immunity tests be carried out, the relation of the virus of herpes zoster to that of herpes simplex remains a matter of speculation. In view of the fact that herpes simplex can be easily and regularly transmitted to rabbits, whereas in the hands of a large number of investigators similar experiments with herpes zoster are completely negative, it does not seem likely that the etiological agent concerned in these two diseases can be absolutely identical.

The question of the identity or non-identity of herpes zoster and varicella is even more difficult to answer, because at present neither

of these infections is readily transmissible to animals. The work of Kundratitz is extremely interesting. His observations, aside from indicating a close immunological relationship between herpes zoster and varicella, are important in that they seem to show the presence of a transmissible virus in the vesicles of herpes zoster. The only question that arises is whether the cases of herpes zoster from which Kundratitz was able to make successful transfers were true cases of idiopathic herpes zoster.

## CONCLUSION.

Attempts to inoculate rabbits, guinea pigs, and monkeys with material obtained from nine cases of herpes zoster have proved unsuccessful.

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